P-064	**Membrane emulsification for the fabrication of microstructured multifunctional emulsion droplets**	

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INTRODUCTION AND OBJECTIVES

Simple and multiple emulsions are functional multi-material structures with applications in different industrial sectors such as chemical, biomedical, biotechnology, food and home and personal care (HPC). They can be used to encapsulate hydrophilic or hydrophobic compounds providing high capacity of entrapment, protection of fragile substances, a combination of incompatible substance in one product and controlled release.
Most conventional methods for making microstructured materials involve drop breakup using shear or impact stresses generated by mechanical agitation. However, such stresses are not uniform across the system, nor are they finely controlled. Dispersed materials produced in these ways thus consist of drops that are highly polydisperse size. In addition, bioactive ingredients like proteins can lose their functional properties.
Membrane emulsification is a high throughput micro-manufacturing processes that allow design microstructured multi-material components with target particle size and size distribution, complex 3D structures, encapsulated formulations etc [Piacentini, 2010; Giorno, 2008; Shah, 2008]. Membrane processes has unique feasibility for formulations containing bioactive labile molecules and structures, and are also among the most sustainable, clean and safe manufacturing processes. They offer an alternative and versatile route to produce dispersed materials by precisely fabricating one drop at a time at low shear conditions.
In this work, membrane emulsification is proposed as advantages technology to functionalize microstructured interface with labile biocompounds with the aim to prepare emulsions having release properties controlled by external stimuli. A multiple emulsion containing a bio-receptor (Conc A) that specifically recognizes and interacts with an artificial ligand (Glucose) was manufactured by the membrane process and used as a model system. This is the first example biomolecule-responsive emulsions fabrication. In order to prove the concept of using Conc A as glucose sensor in multiple emulsions prepared using membrane emulsification, this work first attempts to:
- formulate a W_1/O emulsion in which Conc A is distributed at the interface using cross-flow membrane emulsification. W_1/O emulsion properties were evaluated.
- formulate $W_1/O/W_2$ emulsion using stirred cell membrane emulsification. $W_1/O/W_2$ emulsion properties were also evaluated.
- verify if the release is controlled by the presence of the stimulus.

MATERIALS AND METHODS

In this work two different membrane emulsification processes are applied. In the first step, cross-flow membrane emulsification is used to prepare a water-in-oil (W/O) emulsion. The W/O emulsion has been prepared using soybean oil (Sigma) as the continuous phase and 0.1 wt % Conc A (from *Canavalia ensiformis* (Jack bean) Type IV, lyophilized powder, Sigma,) dissolved in buffer solution (20 mM Tris HCl (>99%, Sigma), 1 mM $CaCl_2$ (96%, Sigma), 1mM $MnCl_2$ (>99%, Sigma), 1 M NaCl (Sigma)) at pH 5.4 as dispersed phase. Microporous hydrophobic glass tubular membranes were supplied from SPG Technology (Japan) with a mean pore size of 0.4 μm. The dispersed phase contain also (D, L)-Phenylalanine (PhAla, 99%, Sigma) used as an indicator to study and verify the controlled release.
In the second step, a W/O/W emulsion is prepared using stirred cell membrane emulsification. The W/O/W emulsion has been prepared using the W/O emulsion prepared in the first step as dispersed phase. Hydrophilic metallic flat-sheet membrane was supplied from Micropore Technologies Ltd with a mean pore size of 10 μm. The composition of water continuous phase was changed to evaluate the effect on droplets size, droplet size distribution and controlled release. We prepared the multiple emulsion using: 0.2wt % Conc A or 2wt % Tween 80 or 0.2wt % Conc A + 2wt % Tween 80.
The droplet size distribution was determined by a laser light scattering system (Malvern Mastersizer 2000, Malvern Instruments) and optical microscope (Zeiss, model Axiovert 25).
To investigate the controlled drug release properties of multiple emulsions prepared a specific amount of glucose (5g/l) was added to the continuous phase and the amount of PhAla released was analyzed using HPLC method. The concentration of PhAla released was measured by using a CROWNPAK CR (+) column (150mm×4 mm) (Daicel Chemical Industries, ltd.), a mobile phase made of $HClO_4$ pH 7 flowed at 0.8 ml/min, 200 nm, 25 °C.

RESULTS AND DISCUSSION

Conc A showed emulsifier property in the W_1/O emulsion prepared in the controlled shear stress conditions using crossflow membrane emulsification. However, results shows that the combination of Conc A and mono-

meric emulsifier added in the oil phase (Span 80) influence emulsion stability (Table 1).

Table 1 W_1/O emulsion prepared by cross-flow membrane emulsification

Emulsifier	w/o %	w/o % After 70 h	D[3,2] (μm)	Span
0.1 wt % Conc A	3	0.6	7.4	1
0.1 wt % Conc A + 2%wt Span 80	3.2	1.5	6.7	1

The experimental results obtained in the preparation of $W_1/O/W_2$ emulsion are given in Table 2. When Conc A was used, droplets with a larger mean particle diameter were obtained due to the higher molecular weight and lower flexibility of proteins. Emulsions prepared using monomeric emulsifier (like Tween 80) have a narrower droplet size and smaller droplet size distribution compared with other prepared emulsions. The presence of Span 80 in the oil showed an additive effect with Tween 80 and Conc A in the continuous phase and both mean particle diameter and span decrease.

Table 2 $W_1/O/W_2$ emulsion prepared by stirred membrane emulsification

W_1/O emulsion emulsifier	$W_1/O/W_2$ emulsion emulsifier	o/w %	D[3,2] (μm)	Span
0.1 wt % Conc A	0.2 wt % Conc A	10	92	1.3
0.1 wt % Conc A	2 wt % Tween 80	10	53	0.9
0.1 wt % Conc A	0.2 wt % Conc A + 2%wt Tween 80	10	63	0.6
0.1 wt % Conc A + 2%wt Span 80	0.2 wt % Conc A + 2%wt Tween 80	10	56	0.8

In order to evaluate Conc A glucose sensor property in W1/O/W2 emulsion the release of a marker substance, i.e. PhAla, dissolved in W_1 aqueous phase was followed with time as a function of glucose presence.
PhAla % released as a result of glucose stimulus was calculated as following:

$$\%PhAla = \frac{PhAla_{GLU} - PhAla_{noGLU}}{PhAla_{noGLU}} * 100$$

in which $PhAla_{GLU}$ is the amount of PhAla released in the presence of glucose and $PhAla_{noGLU}$ is the amount of PhAla released without glucose stimulus.
The % PhAla released as a result of glucose stimulus is a function of emulsion interface composition (Fig. 1). In all the considered systems, Conc A was able to release the marker substance as a function of glucose stimulus. In fact, the amount of PhAla released from W1/O/W2 emulsion was greater in the presence of glucose. The high affinity between Conc A and glucose determined a preferential interaction between them, causing the protein displacement from emulsion interface with phase separation and marker substance release.

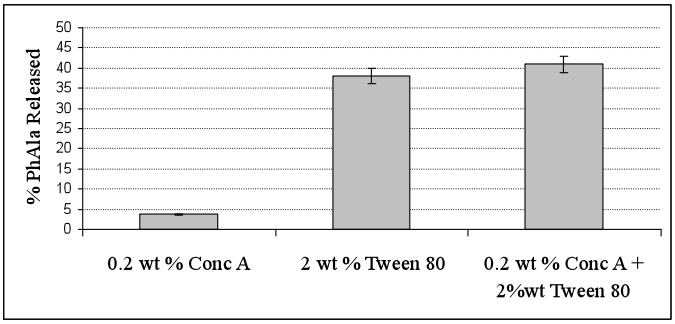

Fig. 1 PhAla % released as a result of glucose stimulus

An additional evidence of glucose stimulus effect in the first system considered was showed in Fig. 2. The progressive addition of glucose in the water phase determines the reactivation of PhAla marker substance release. This proofs the concept that Conc A functions as glucose sensor in the multiple emulsion considered controlling the release as a function of glucose stimulus.

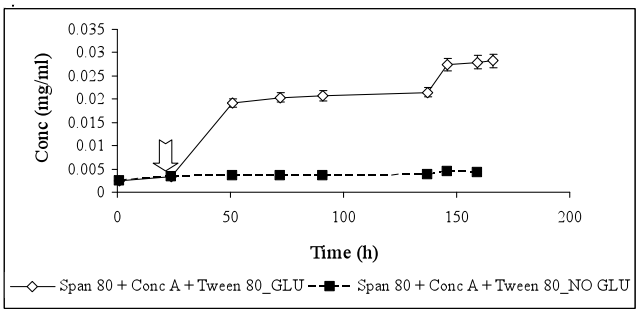

Fig. 2 The effect of progressive addition of glucose in PhAla release

CONCLUSIONS

The work showed that it is possible to use the membrane emulsification technology to produce droplets with specific property and controlled structure using surface-active biosensing molecules in order to obtain biohybrid multifunctional dispersed systems

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